

**STUDIES OF WATER BINDING
IN AMPHIPHILIC SYSTEMS
BY MEANS OF
DEUTERON MAGNETIC RESONANCE**

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av

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STUDIES OF WATER BINDING

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STUDIES OF WATER BINDING IN AMPHIPHILIC SYSTEMS BY MEANS OF
DEUTERON MAGNETIC RESONANCE.

Nuclear magnetic resonance (NMR) provides a convenient non-perturbative technique for studying molecular interactions, which requires resonable amounts of sample and may be very specific. In the field of liquid crystals the many investigations hitherto performed ⁽¹⁾ constitute an excellent complement to the structure determinations made mainly by means of X-ray methods ^(2,3) as the NMR results describe the molecular dynamics and molecular interactions. The present thesis gives a summary of systematic studies of the interaction of water with the amphiphile aggregates in lyotropic liquid crystals, and results from the following papers are treated:

- I. N.-O. Persson and Å. Johansson. Studies of Lyotropic Mesophases Containing Ionic Amphiphiles by Means of ^2H and ^{23}Na Resonance, Acta. Chem. Scand. 25, 2118 (1971).
- II. N.-O. Persson, H. Wennerström and B. Lindman. Counter-ion Dependent Water and Amphiphile Orientation and Deuteron Exchange in Lyotropic Mesophases. Acta. Chem. Scand. 27, 1667 (1973).
- III. H. Wennerström, N.-O. Persson and B. Lindman. Double Quantum Transitions in Deuteron NMR Spectra of Lyotropic Liquid Crystals. J. Magn. Reson. 13, 348 (1974).
- IV. N.-O. Persson, K. Fontell, B. Lindman and G.J.T. Tiddy. Mesophase Structure Studies by Deuteron Magnetic Resonance. Observations for the Sodium Octanoate-Decanol-Water System. J. Coll. Int. Sci. 53, 461 (1975).
- V. N.-O. Persson and B. Lindman. Deuteron Nuclear Magnetic Resonance in Amphiphilic Liquid Crystals. Alkali Ion

Dependent Water and Amphiphile Orientation. J. Phys. Chem. 79, 1410 (1975).

- VI. N.-O. Persson and B. Lindman. ^2H and ^{14}N NMR Studies of Amphiphilic Liquid Crystals. Effect of Solubilisation, Electrolyte and Temperature on Water Orientation. Mol. Cryst. & Liq. Cryst. in press.
- VII. N.-O. Persson, G. Lindblom, B. Lindman and G. Arvidson. Deuteron and Sodium-23 NMR Studies of Lecithin Mesophases. Chem. Phys. Lipids 12, 261 (1974).
- VIII. G. Lindblom, N.-O. Persson, B. Lindman and G. Arvidson. The binding of Water and Sodium Ions in Model Membrane Systems Studied by NMR. Ber. Bunsen Ges. 78, 955 (1974).

The water interactions are studied for D_2O containing mesophases by measuring the deuteron quadrupole splittings; some complementary information has been obtained from counter-ion NMR measurements. Studies of the latter have been performed for a long time in this laboratory ^(4,5,6,7). Below a brief presentation of the lyotropic mesophases with the emphasis on water interactions and a short review of the theory of quadrupole splittings are given prior to a discussion of the results obtained.

Amphiphilic Molecules and Lyotropic Mesophases.

An amphiphilic molecule consists of a hydrophobic part, mostly one or more hydrocarbon chains and a hydrophilic part consisting of an ionic, zwitterionic or nonionic polar group. Typical examples of amphiphilic substances are fatty acid soaps, n-alkylammonium halides and nonionic emulsifiers of the alkyl polyether type. This molecular structure promotes the well

known self-association in aqueous solutions into minor aggregates at lower concentrations^(8,9), and then over a certain concentration, the critical micelle concentration (CMC), bigger aggregates of typically 50-200 monomers, called micelles, appear⁽⁸⁾. The first formed micelles are mostly of globular shape with a hydrocarbon interior surrounded by the polar groups, to which the counter ions are to a certain degree associated. The polar groups are more or less hydrated as are the counter ions.

One of the most remarkable properties of the micelles is the solubilisation phenomenon,⁽¹⁰⁾ i.e. organic substances usually insoluble in water are incorporated in the liquid-like interior of the micelles, and thus the solubilities of such substances in water are greatly increased by the presence of the amphiphile. Also substances with a hydrocarbon chain and a weakly polar group, an aliphatic alcohol or a fatty acid for example, which by themselves do not aggregate into micelles and thus have low solubilities in water, can be solubilized thus forming mixed micelles with the solubilizing agent. Such substances then have their polar groups exposed to the aqueous environment. The two solubilisation modes described above seldom appear in pure form, the practical case will be an intermediary one.

If the concentration of amphiphile is increased the shape of the micelles may be altered from globular to markedly anisometric oblate (disc-shaped) or prolate (cylindrical)⁽⁸⁾ aggregates. Further addition of amphiphile leads to the separation of a mesophase mostly of the normal hexagonal type⁽¹¹⁾. Also addition of a sufficient amount of solubilisate may lead to the separation of various mesophases. The complicated phase equilibria in ternary systems consisting of water, amphiphile and a third component have been thoroughly studied by Ekwall and collaborators^(11,12) and also by others⁽¹³⁾. Figure 1 shows the phase diagram of a typical system of this kind with some phase structures inserted.

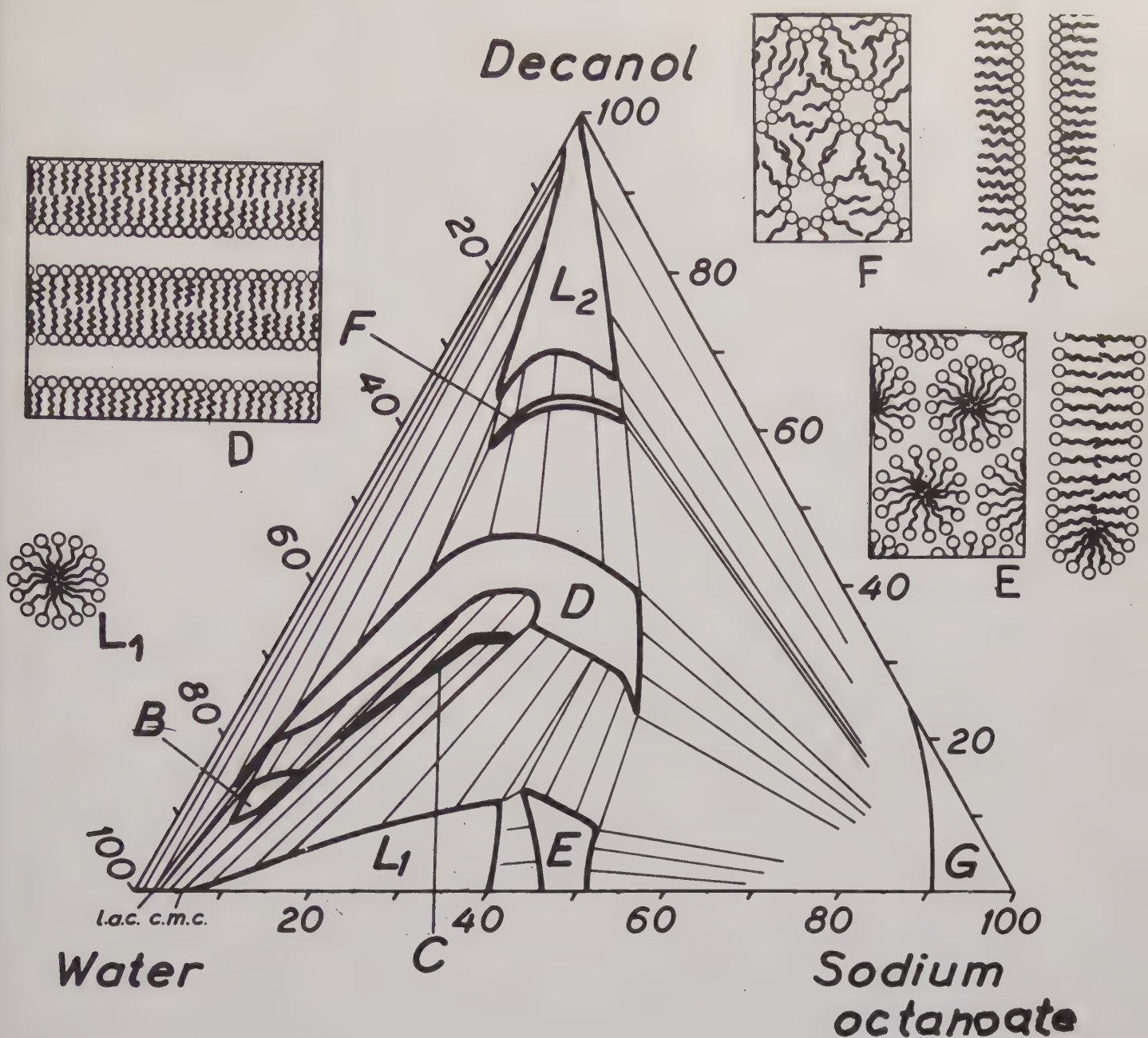


Fig. 1. Phase diagram of the ternary system sodium octanoate-decanol-water redrawn after Ekwall et al.⁽¹²⁾. Schematic structures of micellar solutions and some liquid crystalline phases are inserted. L_1 and L_2 denote normal and reversed micellar solutions, respectively, and D , E and F are the lamellar, normal hexagonal and reversed hexagonal mesophases, respectively.

In these systems two kinds of isotropic solutions are often encountered, the normal aqueous one discussed above and one solution in organic medium where often reversed micelles appear, i.e. micelles with a water core surrounded by the polar groups and the amphiphile hydrocarbon chains forming part of the organic continuum⁽¹⁴⁾. The structures of the various mesophases found in these systems have been elucidated by means of X-ray^(2,3) and electron microscopy⁽¹⁵⁾ techniques. The most thoroughly characterized lyotropic mesophases are the normal hexagonal, the reversed hexagonal and the lamellar. The hexagonal mesophases are built up of indefinitely long cylinders ordered in a two-dimensional hexagonal lattice. The terms normal and reversed have the same meaning as above; in a normal structure the amphiphile aggregates are surrounded by a water continuum, in a reversed structure the opposite pertains. The lamellar phase consists of alternating sheets of water and hydrocarbon chains of indefinite extension in two dimensions separated by the amphiphile polar groups. A variety of other phases has been proposed by many authors^(3,11,16), but the structure and/or existence of some of them have been questioned^(17,18,19,20,IV). The molecular mobilities in both the hydrocarbon and water compartments of all mesophases are rapid in certain directions but strongly hindered in others. The hydrocarbon chains are flexible^(3,11), there is rapid translational diffusion of amphiphile molecules along the aggregate surfaces^(20,21) and water molecules not engaged in hydration of polar groups or counter ions can be considered to have properties approaching those of the pure liquid.

The dimensions of the amphiphilic spheres, cylinders or layers are mostly slightly shorter than twice the length of the extended hydrocarbon chains. On the other hand, the dimensions of the water compartments may vary considerably between different phases or with composition for a single phase. The aim of the

present investigation was to provide information on the binding state of water, i.e. the binding to the aggregate surfaces, in some of the mesophases described above. Most interest was devoted to the lamellar phase due to its existence over wide range of water concentration in different systems⁽¹¹⁾ and the general view that it represents a simple model of biological membranes⁽³⁾, but also the normal hexagonal phase has been studied extensively.

Survey of Studies of Water in Lyotropic Mesophases.

It was realized at an early stage that the role of water in lyotropic liquid crystals is far from that of an inert solvent in which the amphiphilic aggregates are formed^(11,14). Instead there is an interaction of the water molecules with amphiphile polar groups and counter ions and this may be of great importance for aggregate shape and solubilisation properties⁽¹⁴⁾. It is also evident that the dimensional restrictions in one or two directions imposed on the water compartments will give this water other properties than those of bulk liquid water. Spectroscopic studies in the near IR^(22,23), which provide possibilities of determining the fraction of water molecules taking part in hydrogen bonds, have shown that this fraction is increased in liquid crystals. Studies of the water vapour pressure over various amphiphilic solutions and liquid crystalline phases⁽²⁴⁾ have revealed that this quantity is usually markedly depressed for the lower water concentrations; this tendency is best visible within the D- phase domain.

The most common way of discussing water binding in lyotropic liquid crystals is to distinguish only between two kinds of water molecules^(17,22,24); firstly "bound" water molecules which interact with the polar groups of the amphiphile aggregates and have restricted molecular motion, and secondly "free" water

molecules which are situated between the layers of bound water and behave nearly as bulk liquid water. The individual molecules are usually considered to exchange rapidly between the two categories (cf. below).

Self-diffusion studies, both with radiotracer techniques^(25, 26) and pulsed gradient NMR^(27,28), have shown that the self-diffusion coefficient of water in lamellar mesophases is in the range 10^{-10} - 10^{-9} m²/s. This is not far below that of pure liquid water⁽²⁹⁾ and indicates a liquid-like state of the water.

The first NMR investigation of lyotropic liquid crystals was a proton study performed by McDonald⁽³⁰⁾. He found that with a high resolution spectrometer only the water signals could be observed owing to the higher average mobility of the water molecules. Another proton magnetic resonance investigation concerning water molecules in lamellar lyotropic liquid crystals has been carried out by Tiddy⁽¹⁷⁾ in the system sodium octanoate-decanol-water. The spin-lattice relaxation time (T_1) of water indicated fast exchange (i.e. short residence time in each site on the relevant time scale, cf. below) between water bound to the amphiphile and free water, the fraction of the latter decreasing with decreasing total water mole fraction in the samples.

A convenient way to eliminate the disturbing influence of the alkyl chain protons when studying water in the lyotropic mesophases by NMR is to replace water with heavy water and record the deuterium signal. The deuterium nucleus also has the advantage of possessing a quadrupole moment (for details cf. below). Measurements of the thus resulting quadrupole splittings are informative as regards the orientation of water molecules in lyotropic liquid crystals. The first application of this technique was performed by Lawson and Flautt⁽³¹⁾. Later

contributions have been made by Charvolin and Rigny⁽³²⁾, Johansson and Drakenberg⁽³³⁾, Salsbury, Darke and Chapman⁽³⁴⁾, Finer and Darke⁽³⁵⁾ and others. In every case it was noticed that the water molecules are partially oriented. Before discussing the results of this thesis a brief account of the theory of heavy water deuteron splittings will be given following the discussion in ref. (36). As NMR measurements of counter ions are valuable tools for the understanding of the situation on the aggregate surfaces, also the observable parameters in this case⁽⁶⁾ are also mentioned.

Nuclear Quadrupole Effects in NMR-spectra of Liquid Crystals

General Theory

Every nucleus with the spin quantum number (I) greater than $1/2$ has a quadrupole moment. Interaction of the latter with an inhomogeneous electric field at the nuclear site causes shift up or downwards of the $2I + 1$ Zeeman energy levels of the nucleus in a magnetic field⁽³⁷⁾. Thus the NMR absorption is split into $2I$ equidistant bands whose separation is dependent on the magnitude of the quadrupole interaction. A fundamental property of this interaction is that it varies in a significant way with the orientation of the molecule relative to the magnetic field direction. As a consequence of this the static quadrupole interactions are averaged out for the case of fast motion of the molecules in an isotopic environment and no splitting is observed. On the other hand the time fluctuations of the interactions (dynamic effects) will cause nuclear magnetic relaxation and thus broadening of the single band. In a rigid solid the spectrum will mostly be dominated by static effects and a splitting appears.

Molecules in liquid crystals possess an intermediate mobility; the translational velocity in certain directions is of the same order of magnitude as in a liquid, but owing to the long-range order of the amphiphilic aggregates it will be anisotropic and a residual quadrupole splitting is observed. Detailed theoretical analysis of these phenomena have been performed by many authors (6,36,38-40). Here only a very brief summary of the derivations is presented and the final results are given and commented on under the separate paragraphs on static and dynamic effects below.

In the nuclear spin Hamiltonian the quadrupolar interaction is considered as a sum of products of the irreducible tensor components of second order of the electric field gradient at the nuclear site and the irreducible components of a second order spin tensor operator⁽⁶⁾. As the former are best given in a molecular coordinate system, while the latter are most conveniently expressed in a laboratory frame (owing to the Zeeman interaction), a coordinate transformation of the electric field gradients will be necessary. The long range order of the phases makes it suitable to perform this transformation from the molecular to the laboratory coordinates via a so-called director coordinate system. The director axis is usually chosen identical with the optical axis of the phase and the nuclear environments are considered to be of cylindrical symmetry around the director. The transformation of coordinates is expressed by means of Wigner rotation matrix elements, and in the final equations all time dependence of the quadrupole interactions is contained in the latter.

To separate static and dynamic effects, the quadrupole interaction is expressed in one time averaged part and one time fluctuating (which averages to zero) which accounts for the splitting and the relaxation effects respectively. It is also assumed that a certain nucleus has the same direction of the director axis for a long time compared to the NMR-time-scale which implies that all the time dependence of the quadrupole

interaction lies in the Wigner rotation matrix elements specifying the rotation around the director axis.

Nuclear Quadrupole Splittings

If the derivations indicated above are performed in detail for a nucleus with $I = 1$, i.e. for inter alia ^2H or ^{14}N in a sample with a uniform director orientation a spectrum consisting of two absorptions is obtained with a separation in frequency units⁽³⁶⁾:

$$\Delta(\theta_{\text{LD}}) = \left| \frac{3}{4} \frac{e^2 Qq}{h} \cdot S \cdot (3 \cos^2 \theta_{\text{LD}} - 1) \right| \quad (1)$$

The two bands are situated symmetrically around the Larmor resonance frequency. Analogously three lines are obtained for a nucleus with $I = \frac{3}{2}$ (e.g. ^{23}Na) with the separation

$$\Delta(\theta_{\text{LD}}) = \left| \frac{1}{2} \cdot \frac{e^2 Qq}{h} \cdot S \cdot (3 \cos^2 \theta_{\text{LD}} - 1) \right| \quad (2)$$

In these formulae $\frac{e^2 Qq}{h}$ denotes the quadrupole coupling constant of the nucleus, where eq is the principle tensor component of the electric field gradient at the nuclear site and eQ is the quadrupole moment of the nucleus. θ_{LD} is the angle between the director axis and the magnetic field and S the so-called order parameter which is given by:

$$S = \frac{1}{2} \left\{ \overline{(3 \cos^2 \theta_{\text{DM}} - 1)} + \eta \overline{(\sin^2 \theta_{\text{DM}} \cos 2 \phi_{\text{DM}})} \right\} \quad (3)$$

Here η is a parameter that characterizes the deviation from cylindrical symmetry of the electric field gradient and θ_{DM} and ϕ_{DM} are equal to the Eulerian angles β and γ respectively in the coordinate transformation from the director to the molecular systems⁽⁶⁾. The relations between the laboratory, director and molecular coordinate systems is visualized in fig. 2.

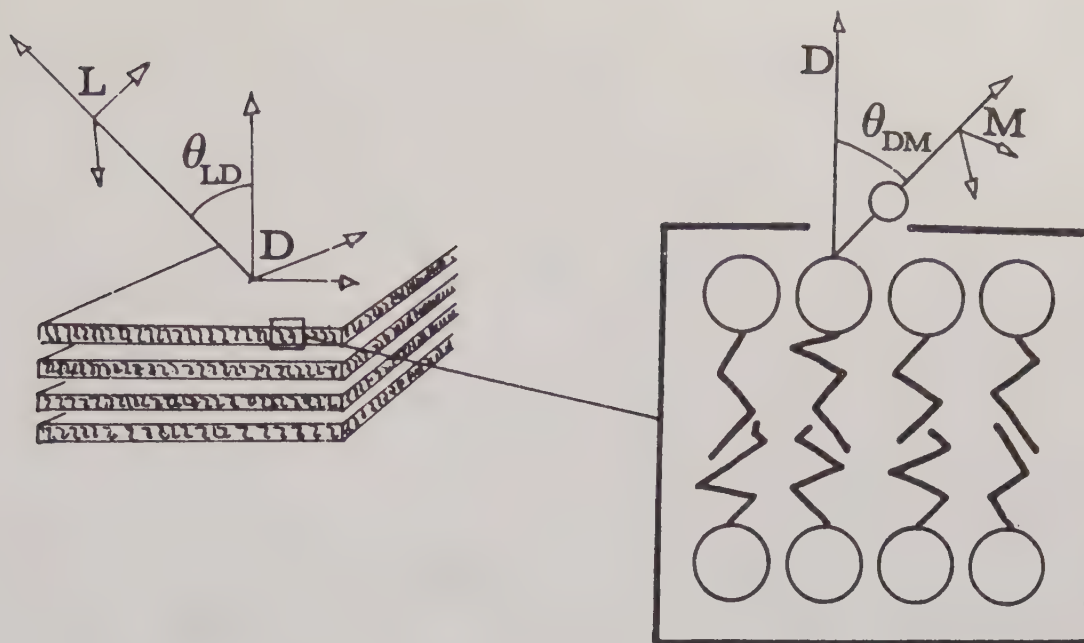


Fig. 2. Schematic representation of the laboratory, director and molecular coordinate systems dealt with in the theoretical treatment of quadrupole splittings. For details cf. text.

From ref. 6.

In a non-oriented liquid crystalline sample the angle θ_{LD} takes any value between 0 and π adians with a spherical probability distribution and thus "powder pattern" spectra (Fig. 3) are obtained.

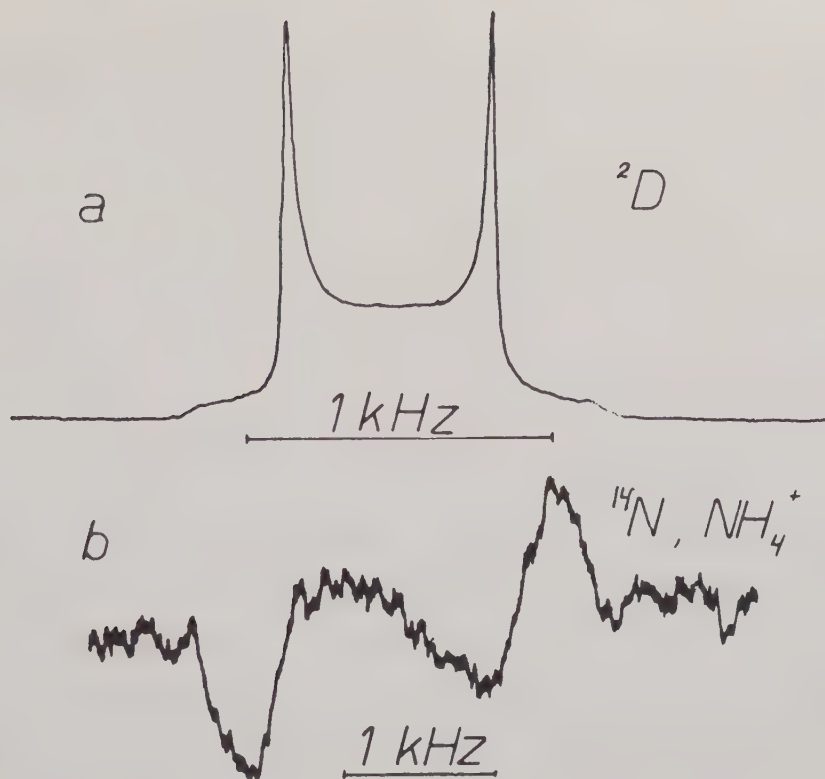


Fig. 3. Experimental quadrupole splitted NMR spectra for nuclei with $I=1$ in powder samples: a) Pulse Fourier transform ^2D -spectrum of D_2O in a normal hexagonal mesophase, which shows a typical powder pattern. b) Continuous Wave wide-line ^{14}N spectrum of a lamellar mesophase which is a derivative of the above pattern.

The separation between two adjacent maxima equals that when $\theta_{\text{LD}} = \pi/2$ radians in the above formulae (1) and (2). In the case of a nucleus with $I = \frac{3}{2}$ the two near-lying Zeeman energy levels where the magnetic quantum numbers (m) are equal to $-1/2$ and $+1/2$ have equal quadrupolar shifts and hence the central band, corresponding to the transition between these energy levels,

is not shifted (to first order) and thus this band remains sharp also in the powder spectra. This fact is used in linewidth studies of ^{23}Na relaxation (cf. below).

It is evident from the above formulae that the observed splitting in a powder sample will be dependent on two factors: the nuclear quadrupole coupling constant and the order parameter. In the case of deuterium in D_2O a separation of these two factors can be made rather reliably as the field gradients are of predominantly intramolecular origin. The nuclear quadrupole coupling constant of deuterium in liquid D_2O has been estimated as 220 kHz⁽⁴¹⁾. Only moderate variation in this parameter owing to varying degree of hydrogen bonding⁽⁴²⁾ has been considered to occur. In the case of ^{14}N in NH_4^+ -ions or ^{23}Na in Na^+ -ions the field gradients are of tetrahedral and spherical symmetries, respectively, in the free ions and the components of the field gradients then cancel and no splittings are observed. Thus, when a splitting does appear this is the result of intermolecular interaction with the aggregate surfaces, and it is difficult to separate the splitting into one factor from the nuclear quadrupole coupling constant and another from the orientation parameter (S).

From eq. (3) it is evident that the magnitude and sign of the S-parameter are strongly dependent on the range of the angle θ_{DM} . At a fixed θ_{DM} at 54.77° a zero value is obtained, provided $\eta = 0$ and then no splitting is observed. Thus it is not only the degree, but also the mode of orientation that determine the observed quadrupole splitting.

b. Quadrupole relaxation

The dominant relaxation mechanism for a quadrupolar nucleus is often (for diamagnetic systems) fluctuations in the quadrupolar interactions due to molecular rotation or translation. The simplest case of quadrupolar relaxation is when "extreme narrowing"

conditions can be assumed, which occurs when $\omega_0 \tau_c \ll 1$. Here ω_0 is the angular Larmor resonance frequency of the nucleus (radians per sec.) and τ_c is the correlation time for the fluctuation⁽⁴³⁾. In this case the transverse (T_2) and longitudinal (T_1) relaxation times are equal and their inverses amount to:

$$\frac{1}{T_1} = \frac{1}{T_2} = \frac{3 \cdot (2I+3)}{40I^2(2I-1)} \left(1 + \frac{\eta^2}{3}\right) \left(\frac{e^2 q Q}{h}\right)^2 \cdot \tau_c \quad (4)$$

The symbols used are defined as earlier. The unsplit spectral line has a characteristic shape termed Lorentzian and the width at half height in Hz is proportional to the inverse relaxation time

$$\Delta\nu_{1/2} = \frac{1}{\pi T_2} \quad (5)$$

If extreme narrowing conditions do not apply the relaxation times are not well-defined for nuclei with $I > 1$ ⁽⁴⁴⁾. In the studies in this thesis the ^{23}Na relaxation is assumed to obey extreme narrowing conditions, which has also been assumed in other studies⁽⁴⁾.

Double Quantum Transitions

When the deuterium spectrum of a lyotropic liquid crystal containing D_2O besides the ordinary powder pattern spectrum also exhibits a sharp central peak between the powder pattern maxima, two explanations come to mind; either there are two sites of deuterons under slow exchange conditions (cf. below), one of which has no splitting⁽³²⁾, or the sample is inhomogeneous i.e. there are two phases, one of which is characterized by water molecules in isotropic environments. In paper III an alternative explanation of the appearance of a central peak is given in terms of double quantum transitions. Such transitions

imply, for a deuterium nucleus, a transition between the Zeeman energy levels characterized by $m=1$ and $m=-1$ under the absorbance of two radiation quanta. As the shift of these energy levels by quadrupolar interaction is the same for all values of θ_{LD} , the absorbed radiation will be at the Larmor frequency for any value of θ_{LD} and a sharp relatively intense peak is observed. Such transitions can only be seen when the radiofrequency field is sufficiently strong. By comparing the experimental lineshapes with computer simulated ones for double quantum transitions from the lineshape equations given by Yatsiv⁽⁴⁵⁾ and refined by Hoffman⁽⁴⁶⁾ satisfactory agreement was obtained. The saturation behaviour, i.e. the effect on broadening and intensity of the spectral components of increasing the intensity of the radiofrequency field, was also studied and was found to be quantitatively in line with the theory.

Thus the possibility of double quantum transitions should be considered when central peaks appear in deuterium resonance especially when high radiofrequency fields are used. For a nucleus with $I=3/2$ the presence of double quantum transitions will give apparent splittings amounting to half the real value⁽⁴⁷⁾. A fast and reliable check on the presence of double quantum transitions is given by pulse-Fourier NMR as in this case the signal is observed after the radiofrequent field is switched off⁽⁴⁸⁾, and thus no double quantum transitions can occur. Recently Mely and Charvolin⁽⁴⁹⁾ have concluded from pulse-NMR measurements that the central peaks formerly observed⁽³²⁾ in some of their deuterium spectra should be ascribed to double quantum transitions.

Chemical Exchange Phenomena

The discussions above are valid only if there is just one kind of site for the studied molecule or ion in the sample.

In an amphiphilic mesophase there are often several sites with very different orientation properties, between which the molecules or ions are exchanging. In considering the effect of chemical exchange rate on the quadrupole broadened or quadrupole splitted spectra it is convenient to discern three categories:

i) When the residence time of the appropriate molecule or ion in a site is more than one order of magnitude longer than the inverse of the difference in the splitting or linewidths between the sites the slow exchange limit applies. Two or more superimposed spectra are then obtained and exchange effects are negligible. ii) When, on the other hand, the residence time is at least one order of magnitude shorter than the inverse of the difference in NMR parameters, then one is dealing with the fast exchange situation. In this case only one well defined quadrupole splitting or Lorentzian line is observed and the splitting amounts to:

$$\Delta_{\text{obs}} = \left| \sum p_i \cdot \nu_{Q_i} \cdot S_i \right| \quad (6)$$

where Δ_{obs} is the observed value of the splitting, Δ_i is the value of this parameter for site i and p_i is the fraction of nuclei in site i . The absolute sign is necessary as the sign of S_i and ν_{Q_i} may be different in different sites. iii) In the intermediate case the lines are broadened in a complicated manner, which deserves detailed theoretical analysis⁽⁵⁰⁾.

The simplest case of exchange considering heavy water in liquid crystals is the concept of "bound" and "free" water molecules mentioned above. As only one well defined quadrupole splitting from the water molecules is observed in a homogeneous phase, the exchange rate between these sites must be fast. A more detailed discussion of these two water molecule sites and

their properties will be given in a subsequent paragraph.

Another complication concerning deuteron quadrupole splittings, first discussed by Ellis et al.⁽⁵¹⁾, is deuteron exchange between D_2O and the amphiphile polar groups. Examples of such polar groups are $-ND_3^{+(33,52)}$, $-COOD^{(V)}$ and $-OD^{(II,V,51)}$. Exchange rates vary with pH^(33,52,II,V) and temperature^(32,II). The phenomenon was encountered in paper I and in II a theoretical analysis of such exchange was carried out and experimental data for the D_2O -lithium octanoate-decanol system was evaluated. The exchange rate of deuterons between the decanol $-OD$ group and D_2O was enhanced by rising the temperature from slow (at $20^\circ C$) over intermediate towards fast (at ca. $70^\circ C$). At lower temperatures a large splitting of ca. 20 kHz was observed besides the ordinary water signal, and therefore it was ascribed to decanol deuterons. The theoretical approach in calculating lineshapes was based on the secular approximation (i.e. the transitions $m=1 \rightarrow m=0$ and $m=0 \rightarrow m=-1$ are assumed to be independent). It was also assumed that exchange between sites with different values of θ_{LD} can be neglected. (This would mean exchange between microcrystallites or motion along strongly curved lamellae, for example.) The theoretical lineshape equations given by Binch⁽⁵⁰⁾ were used for both transitions independently, integrated over all values of θ_{LD} and at the final stage the two signals obtained from the independent transitions were added. A good qualitative agreement between theoretical and experimental lineshapes was obtained but it was found difficult to make a fully quantitative analysis, probably owing to spectral intensity problems. It was also found that an increase in pH of the phase rendered the exchange fast, and that replacing the lithium with other alkali metal ions caused only moderate quantitative alterations of the behaviour.

A procedure to calculate the real water quadrupole splittings (i.e. correcting for exchange effects) in these samples was also

delineated. This was accomplished by addition of base until fast exchange was achieved, measuring the decanol deuteron splittings in samples without base and the splittings from the basic samples and use of eq. (6) above. In paper V water deuteron splittings thus obtained are given for different alkali ions in the alkali octanoate-decanol- D_2O systems and it is evident that they are not very different from the directly observed splittings with the possible exception of sodium containing samples. The effect of counter ions on water orientation will be discussed below.

The deuteron exchange between the $-COOD$ group and heavy water for systems composed of octanoic acid alkali octanoate and D_2O is fast and only one well defined splitting is seen. From a comparison of the variation in observed splitting with alkali metal ion (cf. below) with that of the decanol containing systems and the variation in splitting with the ratio of octanoic acid and water it was concluded in paper V that the signs of the splittings of the $-COOD$ and D_2O deuterons are different and then the differences of the contributions from the two sites are observed according to eq. (6).

Phase Structure and Phase Equilibria

The averaging out of the quadrupole splitting is dependent on the anisotropy of the molecular motion over an appropriate time interval. This anisotropy is, owing to the normally relatively rapid translational diffusion along the aggregate surfaces⁽²¹⁾, not only dependent on the strength and mode of the binding of the molecules in question, but also on the aggregate shape. Thus it can be theoretically shown⁽⁶⁾, that the splitting from a molecule oriented in a lamellar phase is twice that of the same molecule in a hexagonal phase provided the orienting

conditions (i.e. molecular interactions etc.) are the same. This seems to hold in some studies by other authors⁽³²⁾ but for a number of systems studied by us considerable deviations from this relationship are obtained, and in paper V possible reasons for this behaviour are discussed. It is argued that an inability of the D_2O molecules to circumvent the cylinders of the hexagonal phase in the relevant time interval should require unrealistically small diffusion coefficients. The presence of curved lamellae which would reduce the splittings of the lamellar phase can also be ruled out after comparison between splittings of lamellar phases oriented between glass plates⁽⁵³⁾ and powder samples. The remaining explanation is that the binding conditions for the water molecules on the aggregate surfaces are altered considerably on going from one phase to another. This hypothesis is supported by X-ray results showing marked variations in the surface per polar group between the phases^(3,11,54,55). It is considered that splittings from deuterons in the amphiphile molecules should provide a more reliable tool for structure determinations.

The effect of phase inhomogeneity on deuterium spectra has been mentioned above in connection with chemical exchange and double quantum transitions. Thus for an inhomogeneous sample two superimposed spectra are observed, e.g. a mixture of isotropic solution and liquid crystal shows a narrow central peak and one splitting, a mixture of two liquid crystalline phases or one liquid crystal and one gel phase show usually two splittings and so on.

The structure of the C-phase (in Ekwall's nomenclature; see Fig. 1), which appears in some ternary systems⁽¹¹⁾, has been described as having a structure consisting of long square amphiphile rods with water between them. However, its existence as a separate phase was questioned by Tiddy⁽¹⁷⁾ who on the basis of his proton NMR measurements in the H_2O -sodium octanoate-decanol system suggested it to be a "flocculated emulsion of lamellar D-phase

in a small amount of micellar solution". Tiddy also supposed that the results obtained by Ekwall et al.⁽¹²⁾ could be interpreted in the same way. The behaviour proposed by Tiddy would according to the preceeding discussion give rise to a deuterium NMR spectrum consisting of one sharp central peak from the micellar solution and one powder pattern from the lamellar phase. From the investigations presented in paper IV it appears that such spectra do appear for the large number of C-phase samples investigated by us in the same system with H_2O replaced by D_2O . Furthermore the intensity of the two spectral components and the magnitudes of the observed quadrupole splittings are, within experimental accuracy, in agreement with the assumption that the samples lie on tie-lines between the lamellar phase and the normal micellar solution (cf. figure 1). As some of the spectra were observed by means of pulse-Fourier transform NMR the possibility of double quantum transitions is ruled out. Deuterium NMR measurements⁽⁵⁶⁾ on samples deuterated on the hydrocarbon chains give results supporting our conclusions. On the other hand the occurrence of a three-phase triangle L_1+C+D (cf. figure 1) in the phase diagram and also other data give support for the idea that the C-phase exists as a separate phase. The argument of Ekwall⁽¹¹⁾ that in the C-phase there are two kinds of water which are slowly exchanging is hard to combine with our and other studies and also with the size of the observed splittings. The latter also make the structure proposed by Ekwall et al. improbable. From the above considerations it is evident that deuterium resonance provides information on mesophase structure. Thus recently a phase diagram for the dipalmitoyllecithin - heavy water system has been constructed from deuterium NMR - results⁽⁵⁷⁾.

Information on Molecular Interactions from Water Quadrupole Splittings

The degree and mode of heavy water orientation and hence the

deuteron quadrupole splitting in a homogeneous lyotropic liquid crystalline sample are affected by several parameters. The most pertinent ones found in this and other studies are the molar fraction of water, the nature of the counter ion and the amphiphile polar groups, and the temperature of the sample. In the following the results from our investigations are briefly reviewed considering these points.

The Effect of the Water Content

This effect is best discussed in terms of the "free" and "bound" water molecules mentioned above. Assuming that the "free" molecules can rotate more or less isotropically and thus exhibit negligible splitting it is evident that the observed quadrupole splitting should decrease with increasing water content. This behaviour is also experimentally verified in many cases. However, a more quantitative treatment can be performed as outlined in paper V. Thus, supposing that the hydration numbers of the aggregated surfactants and the quadrupole splittings of the D_2O molecules in the various bound sites are not altered as the molar fraction of water is varied, a linear plot would result if the quadrupole splitting is represented as a function of molar ratio of total amphiphile to water. Furthermore, if the splitting of unbound water amounts to zero the straight line would pass through the origin.

It is found that many systems, especially those containing alkanoate ions, do follow the described behaviour for lamellar phases especially at higher water contents, and thus it seems meaningful to interpret the results in terms of bound and free water. The region of the lamellar phase where our model applies best is that of the "expanding" lamellar phase i.e. in the water-rich phase domain where in agreement with the present findings the

lamellar surfaces have been considered to be fully hydrated and additional water to be trapped as free molecules in the middle of the water layers⁽²⁴⁾. For other systems i.e. those with alkyl sulfate on alkyl sulfonate surfactants it was impossible to rationalize the obtained data in this way and this was ascribed to alterations in the intrinsic orientation of bound D_2O or in the number of water binding sites. The model described is also found not to apply for the water poor parts of the lamellar phases in most systems, in part owing to the fact that the water content is insufficient to fill the hydration need of both the surfactant polar groups and the counter ions (cf. also below). The consequence of this is that there will be an increased binding of counter ions to the aggregate surfaces and thus a smaller number of binding sites for water^(I,V). These ideas receive support from ^{23}Na NMR results^(I,7). The limited domains of existence of the normal hexagonal phases preclude similar analyses for this phase but the reversed hexagonal phase of the Aerosol-OT (= sodium di-(2-ethyl-hexyl) sulfosuccinate)^{*} + D_2O system has been shown to give the simple linear behaviour.

Effect of the Counter-Ion

The nature of this effect has partly been described above; there is a competition between amphiphile polar groups and counter-ions for water molecules. Mandell⁽⁵⁸⁾ have emphasized that the hydration requirement of the counter ions is of fundamental importance for the extension of the liquid crystalline phases towards the limits of lowest water content.

^{*}The nomenclature of Aerosol-OT I and II used in paper V is inappropriate as both the chemicals have the same iso-octyl hydrocarbon chain as given above.

They found that in the alkali-octanoate-decanol-water systems the minimum water contents per soap molecule of the liquid crystalline phases parallel the hydration numbers of the counter ion for the Li^+ , Na^+ and K^+ soaps. Our studies^(V) suggest that the counter ion hydration water molecules undergo more or less isotropic motion, except those of lithium ion hydration. In the case of lithium ions with their strong hydration the whole hydrated ion complex was argued to be oriented with respect to the aggregate surface which explains the large splittings observed in the lithium-containing systems. This holds for both carboxylate and sulfate surfactants. The results of addition of inorganic salts presented in paper VI strengthen these hypotheses; addition of NaCl decreases the observed splittings but LiCl increases them. The increase in splitting on going from Na^+ to K^+ or Rb^+ as counter ion is on the other hand explained by the fact that the amount of isotropically reorienting hydration water is lower for the heavier counter-ions.

In paper VI the effect of replacing the alkali metal ions with the tetramethylammonium ion is also studied. The obtained deuteron quadrupole splittings are extremely small and this is probably caused by a hydrophobic effect associated with the proximity of the methyl groups of the counter-ions and the methylenes near the carboxylate groups of the surfactant; the tetramethylammonium ions then block the water binding sites of the surfactant, and make the aggregate surfaces more hydrophobic.

Effect of the Polar Groups

When the amphiphile composition of a ternary system is varied at constant molar fraction of heavy water the deuteron quadrupole splittings often follow complicated patterns changing

with the amphiphiles and the fixed molar fraction of water^(I,V). For example, in the sodium octanoate-octanoic acid-water^(I) and lithium octanoate decanol-water^(V) lamellar phases a maximum of the splittings is observed at certain compositions of the amphiphile lamellae. This may be taken as an indication for complex formation between the polar ends of the amphiphiles. Sodium-23 relaxation measurements strengthen this hypothesis in the former case^(I).

The effect of solubilization of hydrophobic substances such as n-octane or carbon tetrachloride in normal hexagonal phases is described in paper VI. It is found that the deuteron splitting is decreased when the amphiphile is sodium octanoate while it is increased when it is sodium octyl sulfate. This is supposed to reflect a greater tendency to lateral expansion of the sulfate-containing surfaces than of those containing carboxylate. In the latter case the surfaces are thought to be stabilized by water molecules interacting simultaneously with two amphiphile molecules or with amphiphile and counterion, which is also in agreement with ²³Na-studies⁽⁷⁾. The decanol -OD results presented in paper V indicate that no significant difference in the order of the amphiphile lamellae prevails between the sodium octanoate-decanol-water and the sodium octylsulfate-decanol-water systems.

Effect of Temperature

For many cases studied, temperature effects are dominated by exchange phenomena (cf. above) and reliable results on the temperature dependence of water orientation are only directly obtainable if the amphiphile(s) contain no exchangeable deuterons. A further difference between the sodium octanoate and the sodium octylsulfate surfactants is encountered here: the deuteron

splittings of normal hexagonal phases composed of surfactant and water decreases with increasing temperature in the former case but increases in the latter^(VI). This is another indication of a markedly different interaction with water between the two polar groups; in the sulfate case lateral expansion creates better binding conditions for water molecules with increasing temperature, in the carboxylate case the binding sites are less variable. Differences in interaction between carboxylate and sulfate polar groups with counter-ion and water, have also been pointed out on the basis of ^{23}Na -NMR results⁽⁷⁾.

Biological Model Membrane Systems

One of the most exciting subjects in connection with lyotropic liquid crystals is the biological cell membrane. Since the pioneering work of Gorter and Grendel⁽⁵⁹⁾ it has become increasingly evident that biological membranes contain a lipid bilayer matrix in which the proteins and other constituents are embedded. The still surviving alternative hypothesis the "association-induction-model"⁽⁶⁰⁾ seems to have lost ground. The present views of the biological membrane are well described by Singer and Nicholson⁽⁶¹⁾ and is illustrated in fig. 4 which is taken from this article.

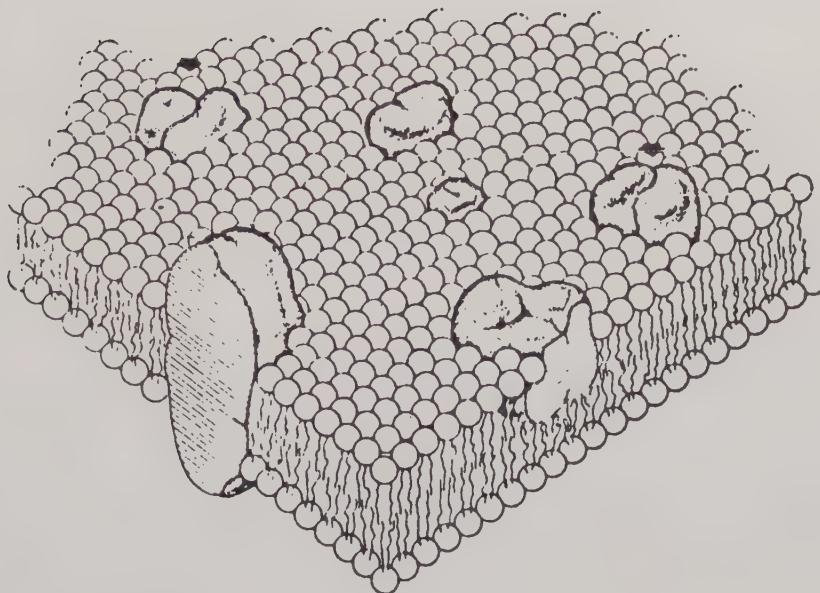


Fig. 4. The schematic structure and organisation of a biological membrane as described by Singer and Nicholson⁽⁵⁹⁾ with the lipid matrix and the proteins more or less completely embedded therein.

The first deuterium resonance study of D_2O in a lipid system with biological connection was performed by Ellis et al.⁽⁵¹⁾ and later Salsbury et al.⁽³⁴⁾ and Finer and Darke⁽³⁵⁾ have presented studies on this subject. In papers VII and VIII some of the contributions from this laboratory to this field are presented.

Liquid crystalline phases in systems of naturally occurring substances, i.e. egg yolk lecithin, sodium cholate and cholesterol mixed with heavy water and NaCl are studied in VII. The phase diagrams of such systems without NaCl have been elucidated by Small⁽⁶²⁾. The most interesting results of our studies are as follows;

- i) Of the alkali metal ions, the potassium ions interact most strongly with the aggregate surfaces.
- ii) Cholesterol addition increases the water deuteron splittings.
- iii) No effect of phase structure on the observed deuteron splittings can be seen.

When the egg yolk lecithin is replaced by saturated lecithin with chemically well-defined fatty acid groups no other alteration in the phase behaviour is observed except for the increase of the phase transition temperature gel $\rightarrow$ liquid crystal to room temperature or thereabove^(57,63). Such systems are investigated in VIII. It is proposed from the results that with increasing temperature or addition of NaCl the choline groups of lecithin are straightened out normal to the surfaces of the lipid lamellae, thus providing more binding sites for water and sodium ions. These ideas have been further developed in a later paper⁽⁶⁴⁾, and similar deductions have been made by other authors in connection with diffusion studies⁽²⁶⁾.

Concluding Remarks

It is evident from our and other studies that heavy water deuteron NMR studies provide good insight into the binding situation of water on the aggregate surfaces in amphiphilic mesophases and biological membranes and also give information

on sample homogeneity. Further information on the details of the molecular interactions could be obtained from ^{17}O resonance studies. The low natural abundance of this nucleus which has $I=5/2$ and thus a quadrupole moment makes the use of this method for powdered samples very difficult, although quadrupole splitted signals have been observed with strongly ^{17}O enriched water⁽⁶⁵⁾. The availability of better NMR instruments and/or higher ^{17}O isotopic proportion in commercially available enriched water will probably circumvent these problems in the near future.

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